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SHORT COMMUNICATION

STANFORD, CALIFORNIA

Tissue Sampling Technique Affects Accuracy of Karyotype from Missed Abortions

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***Purpose*:** To determine if careful specimen selection and washing of tissue from first trimester missed abortion products of conception specimens increases the sensitivity of routine cytogenetics in detecting aneuploidy.
***Methods*:** Retrospective review of cytogenetics results from tissue from dilation and curettage for missed abortion in a university fertility practice between 1998 and 2001. A technique of careful selection and washing of the specimen was implemented in July 1999. Results from before ($n = 15$) and after ($n = 41$) this change were compared. Cytogenetics reports from other physicians using the same laboratory were used for comparison ($n = 59$).
***Results*:** The percentage of 46XX results was significantly decreased in the test group when compared to historical and community controls: 29% vs. 73% and 56% respectively. The percentage of aneuploid results was significantly higher in the test group at 61% vs. 7% and 36% in the historical and community controls respectively.
***Conclusion*:** Thorough separation and cleaning of villi prior to sending missed abortion specimens significantly increases sensitivity of conventional cytogenetics for detecting aneuploidy by decreasing maternal contamination.

KEY WORDS: Cytogenetics; maternal contamination; missed abortion; villi.

INTRODUCTION

Although the loss of a desired pregnancy is disappointing for any patient, the emotional impact of a miscarriage is magnified in the setting of infertility. Physicians and patients may feel pressure to initiate testing for causes of recurrent miscarriages after only one or two losses in an effort to offer specific therapy to prevent future pregnancy losses. Finding an abnormal karyotype in the products of conception (POC) provides an obvious explanation for the miscarriage, which avoids unnecessary testing and treatment. The value of karyotyping on POC is limited by frequent false negative results caused by maternal contamination (1,2). Lowering the false negative 46XX rate would make cytogenetic testing a more appealing option, which would facilitate counseling.

In our infertility practice, we have routinely performed cytogenetic analysis on missed abortions, since 1998. Initially a preponderance of 46XX results was noticed. This prompted us to change our technique for tissue sampling before sending the specimen for evaluation. In this report, we analyze whether careful specimen selection improves the yield of actual fetal karyotypes.

MATERIALS AND METHODS

Since 1998, all patients in the senior author's (A.A. Milki) infertility practice who were diagnosed to have a missed abortion were offered cytogenetic testing of the POC obtained by suction curettage. Testing was performed by the cytogenetics laboratory at our medical center. Prior to July 1999 (Group A, $n = 15$), POC from missed abortions were drained of blood and then divided into a sample sent for histopathologic diagnosis and a sample for chromosomal testing. Since July 1999 (Group B, $n = 41$), the technique for choosing a sample from the tissue for genetic analysis was changed in an attempt to improve diagnostic accuracy. The POC were drained of blood and then placed in a kidney basin containing saline and rinsed thoroughly. They were then placed in a clean saline basin and carefully examined to identify chorionic villi. A sample of villi was dissected clear from other tissue using forceps and scissors and then washed again and sent for chromosomal analysis. There were no significant changes in the demographics of the practice before or after July 1999.

For comparison, we reviewed cytogenetics reports from other physicians using the same laboratory. Cytogenetics results from 59 first trimester missed abortion specimens received after January 2000 were available for review (Group C).

All tissues were submitted to the cytogenetics laboratory in a complete RPMI (Rosewell Park Memorial Institute) media. The tissues were examined routinely and the laboratory staff attempted to choose only villus or fetal tissue to culture. Tissue was cultured and chromosomes were analyzed using the

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GTW banding (G-banding Trypsin–Wright) method. At least 20 metaphases were examined and the final assessment made by the cytogenetics laboratory director was used to finalize the results.

The karyotypes from all three groups were reviewed and compared for the proportion of 46XX, 46XY, and abnormal results. The ages of the women were also recorded and compared. Chi-square, Fisher exact, and Student's *t* tests were used for statistical analysis. Significance was set at 0.05.

RESULTS

The study group had significantly lower rates of 46XX (29%) when compared to Group A (73%) or to Group C (56%). There was no significant difference in 46XX results between Group A and Group C. The percentage of abnormal karyotypes in the study group (61%) was significantly higher than Group A (7%) and Group C (36%). There was no significant difference in the mean age of patients in the three groups (Table I).

DISCUSSION

Conventional cytogenetic analysis of spontaneous abortions depends on tissue culturing and can be associated with significant contamination by maternally derived cells (2). Polymerase chain reaction (PCR) has been used on first trimester abortion specimens to demonstrate that at least 30% of 46XX results are due to maternal cell contamination (1,3). Given the specificity of the probes used for these techniques only errors due to XY fetuses would be recognized and the authors of both studies hypothesized that the true error rate of conventional cytogenetics is even higher because of undetected aneuploidies. We believe that by carefully separating out the villus tissue and thoroughly washing it with saline, maternal contamination of the specimen could be significantly reduced, making genetic testing by cytogenetics of POC a more helpful diagnostic tool in first trimester miscarriages.

The cytogenetics laboratory personnel routinely attempt to isolate villus or fetal material from the submitted sample, which may suggest that careful tissue separation by the clinician is not critical. However, the amount of villus material can be reduced in early first trimester missed abortions and the laboratory may not have the option of culturing the appropriate tissue if the submitted sample is inadequate in that respect. Often in our practice we find that the POC resulting from early nonviable pregnancies contain a very limited amount of villi. These can be easily missed with routine automatic specimen division between pathology and cytogenetics even if a significant portion of the tissue available was sent for karyotyping. The tissue sorting technique we describe significantly decreases the percentage of 46XX results and improves the yield of aneuploidies compared to both historical control patients treated by the same physician as well as those treated by other physicians in the same community. It is possible that the community controls have a different occurrence of cytogenetic abnormalities due to differences in patient population. However the author's historical controls represent the same patient mix as the study population and only differ from the study group with regards to the tissue sampling technique applied. It would be ideal to design a prospective randomized study to better evaluate the efficacy of this technique. It may be more practical, as an intermediate step, to assess the change in the future results of community controls if physicians were to adopt the sampling technique described in this report.

There are several advantages to an accurate cytogenetic evaluation of the POC. Studies on patients with first trimester miscarriages have shown that the pregnancy following a euploid spontaneous abortion is significantly more likely to miscarry than a pregnancy following an aneuploid loss (4–6). Thus decreasing

Table I. Cytogenetics Results on Products of Conception in the Study and Control Groups

	Group A (historical controls)	Group B (study group)	Group C (other physicians)
Number of specimens	15	41	59
Number of 46XX[a]	11 (73%)	12 (29%)	33 (56%)
Number of abnormal[b]	1 (7%)	25 (61%)	21 (36%)
Number of 46XY	3 (20%)	4 (10%)	5 (8%)
Age (average ± SD)	37.6 ± 4.0	37.5 ± 3.8	36.8 ± 4.5

[a] B vs. A, $p = 0.005$; B vs. C, $p = 0.014$; B vs. A+C, $p = 0.004$.
[b] B vs. A, $p = 0.0005$; B vs. C, $p = 0.02$; B vs. A+C, $p = 0.003$.

the percentage of false 46XX results will prevent potential concern created by a normal result.

Patients with previous pregnancy losses are often offered testing for antiphospholipid antibodies and sometimes controversial immunologic testing (7). Anticoagulant therapy for positive antiphospholipid antibodies has been substantiated in recurrent pregnancy loss for patients with significantly elevated levels (8). However low positive levels that are frequently seen in patients with a history of one or two pregnancy losses prior to 10 weeks of gestation could be a spurious finding which would be easier to dismiss in the presence of an abnormal karyotype on the POC. Using the same logic, small or moderately sized fibroids may be blamed for fetal losses in the absence of other explanations. Furthermore, controversial immunologic therapies, which may seem attractive to frustrated patients and physicians, can be easily avoided if the cause of the miscarriage is known to be chromosomal, as found in 61% of our study population. Even more importantly, a false negative 46XX result obtained as a result of poor specimen selection often provides false justification for otherwise unindicated treatments.

An additional benefit from accurate karyotyping of POC is pertinent to patients with recurrent pregnancy loss who are undergoing in vitro fertilization. Information about an abnormal karyotype in a previous miscarriage sample may validate the decision to perform expensive preimplantation genetic diagnosis (PGD). A normal result, especially if obtained by a reliable technique, would suggest that PGD is not critical.

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